PLACENTAL-FETAL GLUCOSE EXCHANGE AND FETAL GLUCOSE METABOLISM

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ABSTRACT

Fetal glucose metabolism depends on additive effects of fetal plasma glucose and insulin. Glucose-stimulated insulin secretion increases over gestation, is down-regulated by constant hyperglycemia, but enhanced by pulsatile hyperglycemia. Insulin production is diminished in fetuses with intrauterine growth restriction (IUGR) by inhibition of pancreatic β-cell replication, but not by mechanisms that regulate insulin production or secretion, while the opposite occurs with hypoglycemia alone, despite its common occurrence in IUGR. Chronic hyperglycemia down-regulates glucose tolerance and insulin sensitivity with decreased expression of skeletal muscle and hepatic Glut 1 and 4 glucose transporters, while chronic hypoglycemia up-regulates these transporters. The opposite occurs for signal transduction proteins that regulate amino acid synthesis into protein. These results demonstrate the mixed phenotype of the IUGR fetus with enhanced glucose utilization capacity, but diminished protein synthesis and growth. Such adaptations might underlie childhood and adult metabolic disorders of insulin resistance, obesity, and diabetes mellitus.

Introduction

Glucose is the principal energy substrate for the placenta and the fetus and is essential for normal fetal metabolism and growth. Not surprisingly, therefore, its supply to these tissues is regulated by a relatively complex set of mechanisms that tend to keep its metabolism relatively constant. The first point in this regulation is the maintenance of maternal glucose concentration by increasing rates of maternal glucose production and development of relative maternal glucose intolerance and insulin resistance. The second point is the transfer of maternal glucose to the fetus by the placenta, which is buffered by placental glucose utilization. The third point is the production of in-

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sulin by the developing fetal pancreas, which enhances glucose utilization among the insulin-sensitive tissues (skeletal muscle, liver, heart, adipose tissue) that increase in mass and thus glucose need during late gestation. Glucose uptake into fetal tissues is regulated by glucose transporters that increase or decrease in response to both acute and chronic changes in fetal glucose concentration and conditions of intrauterine growth restriction. At the same time, signal transduction protein regulators of amino acid synthesis into protein are down-regulated, emphasizing that IUGR presents a mixed phenotype, with increased propensity to take up energy substrates, such as glucose, and diminished capacity for protein synthesis and growth.

Methods

The first measurements of glucose metabolism involving the placenta and fetus were done in acute and chronic preparations in pregnant sheep, in which glucose concentration in the maternal circulation was found to be almost invariably greater than that in the fetal circulation (1). Fick principle measurements under chronic, unstressed conditions quantified the net flux of glucose into and across the placenta and showed that the net flux of glucose is from the mother to the fetus and that nearly all of fetal oxidative metabolism (measured as oxygen consumption) could be accounted for by the net supply of glucose (2). The addition of tracer methodology showed that glucose transport across the placenta was bi-directional, requiring, therefore, a three-compartment model that included uptake and transport of glucose by the maternal, placental, and fetal compartments (3). Tracer methodology also showed that under normal conditions, all glucose used by the fetus was supplied by the mother, and allowed quantification of glucose metabolism into various metabolic pathways in both placenta and fetus (4). Substrate-hormone clamp techniques provided insight into the regulation of glucose uptake, transport, and metabolism in the placenta and fetus according to plasma glucose and insulin concentrations (4). Morphological analysis, cell biology, and molecular biology techniques have helped define the regulation of glucose flux across membranes by glucose transporters; metabolism of glucose by enzymes in the glycolytic, oxidative, and glycogen synthesis pathways; and uptake and metabolism of glucose in cells regulated by genes and their proteins in the insulin signal transduction pathway. Models have been developed to determine coordinated but opposite changes in glucose and amino acid metabolism that help explain the unique phenotype of intrauterine growth restriction.

Results and Discussion

I. Placental glucose transport kinetics

Studies in the late gestation pregnant sheep model have shown that placental and fetal glucose consumption rates vary independently of each other and glucose transporter proteins at both maternal and fetal placental membranes demonstrate saturation kinetics (5,6). In these studies, glucose clamp experiments produced steady state net fluxes of glucose into and across the placenta and into the fetus at different concentrations of glucose in the maternal and fetal circulations. Uptake and net consumption of glucose by the uteroplacenta demonstrate saturation kinetics with a maximum glucose utilization rate (V_{max}) of about 0.23 mmol/min. K_m (maternal arterial glucose concentration at $V_{max}/2$) and K_S (maternal arterial glucose concentration at which V_{max} is reached) were 2.8 mmol/L and 8.0 mmol/L, respectively, not significantly different from the same parameters for uterine glucose uptake (7,8). Similar results have been measured in a variety of in vitro perfusion experiments in human placentas, although V_{max} in the human placenta studied in vitro is several-fold higher than in the sheep (6).

Figure 1 summarizes the regulation and quantitative aspects of placental glucose uptake and transport (9). First, uterine, placental, and fetal glucose uptake are directly related to maternal glucose concentration (Figure 1, left panel) (8). Second, fetal glucose uptake is separately regulated by fetal glucose concentration by which a relatively lower glucose concentration in the fetus establishes a larger maternal-fetal glucose concentration gradient and thus increased transfer of glucose to the fetus (Figure 1, middle panel) (8). In contrast, at any maternal glucose concentration, placental glucose consumption is directly related to fetal arterial plasma glucose concentration (Figure 1, right panel) (7). In fact, the fetal side of the placenta has an eightfold greater capacity to take up glucose than the maternal side, so that changes in fetal glucose concentrations have a strong influence on placental glucose flux and metabolism. Independent increases in fetal glucose concentration, regardless of cause or mechanism (e.g., acute hypoxia-ischemia), acts to divert uterine glucose supply to the placenta.

II. Mechanisms of placental glucose uptake and transport

The fetus grows several-fold in size over the second half of gestation as does its absolute rate of glucose utilization. Placental glucose transfer to the fetus, therefore, must increase to meet the increasing met-

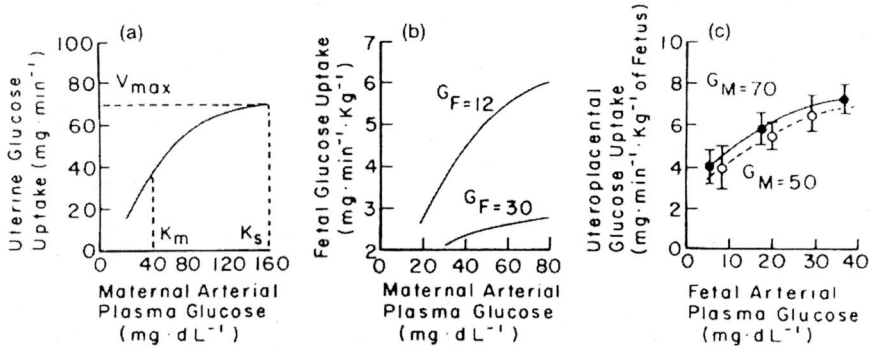

FIG. 1. Uterine, fetal, and uteroplacental glucose uptake rates as functions of maternal (a, b) or fetal (c) arterial plasma glucose concentrations in pregnant sheep (9). Left Panel: Net rate of glucose transfer into the uterus from the maternal plasma versus maternal plasma glucose concentration. V_{max} is the maximum rate that glucose can enter the uterus; K_m is one half of V_{max} and a measure of the sensitivity of uterine glucose uptake to maternal glucose concentration; K_s is the maternal glucose concentration at which V_{max} is reached. Middle panel: Net rate of glucose transfer into the fetus from uteroplacenta versus maternal plasma glucose concentration; a lower fetal glucose concentration relative to any maternal glucose concentration produces a greater maternal-fetal glucose concentration gradient and a higher rate of placental to fetal glucose transfer (upper curve versus lower curve). Right panel: Net rate of uteroplacental glucose consumption versus fetal plasma glucose concentration; at any maternal glucose concentration (data for maternal glucose concentrations of 50 and 70 mg/dL are shown), uteroplacental glucose consumption is directly related to fetal glucose concentration. These data show that maternal glucose concentration determines the rate of glucose entry into the uterus (and thus the fetus and placenta), but the rate of uteroplacental glucose consumption is regulated more by the fetal than the maternal glucose concentration. Reproduced with permission from Hay, W. W., Jr.: Placental Function. In Gluckman, P., Heymann, M. A. (eds.), Scientific Basis of Pediatric and Perinatal Medicine, Second Edition, Edward Arnold Ltd., London, pps 213–227, 1996 (9); originally adapted from Hay WW Jr, Molina RD, DiGiacomo JE, Meschia G. Model of placental glucose consumption and transfer. Am J Physiol 1990;258:R569–R577 (7); Hay WW Jr, Meznarich HK. Effect of maternal glucose concentration on uteroplacental glucose consumption and transfer in pregnant sheep. Proc Soc Exp Biol Med 1988;190:63–69 (8).

abolic requirements for glucose of the larger, growing fetus. The increase in placental glucose transport occurs by two mechanisms: (1) the transplacental glucose concentration gradient increases as the fetal glucose concentration decreases relative to maternal glucose concentration (Figure 2, top panel); (2) the placental transport capacity itself increases (Figure 2, middle panel) (10,11).

A. Placental glucose transport capacity. Placental glucose transport capacity develops primarily by an increase in the total amount of those glucose transporters that are found on the membranes of the uterine endothelium and epithelium, trophoblast, and villous

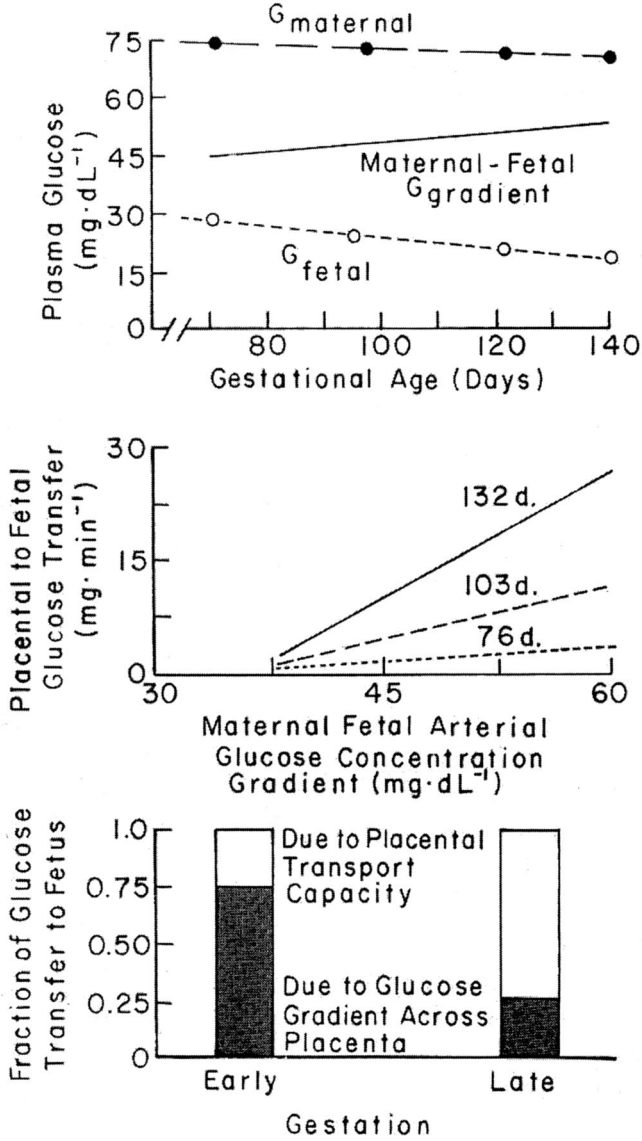

FIG. 2. Plasma glucose concentrations and transplacental glucose transport as functions of gestational age in pregnant sheep (10). Upper panel: Plasma glucose concentration tends to decrease in fetal sheep (-o-) over the second half of gestation relative to the maternal plasma glucose concentration (-•-). This increases the maternal-fetal plasma glucose concentration gradient or the driving force for transplacental glucose transfer (—). Middle panel: Placental-to-fetal glucose transfer (PGT) increases about 8-fold over this same period. This increase in transport largely reflects transport capacity,

endothelium (12). The number of glucose transporter proteins probably increases primarily by an increase in the trophoblast membrane surface area. An increase in the concentration of transporters per unit membrane area also is possible, but not proven. Although there is some evidence from in vitro studies that glucose concentration can regulate placental glucose transporter expression, either directly or through insulin like growth factor 1 (IGF-1) action (11), Glut 1 mRNA may vary independently of Glut 1 protein; for example, Glut 1 protein decreases over gestation in the rat, but it parallels Glut 1 protein in the sheep. There is no evidence, however, that such changes affect the actual rate or the capacity (maximal rate) of placental glucose transport. In fact, in vitro perfusion studies in human placentas and in vivo studies in humans and sheep indicate that placental glucose transport capacity is much greater than actual transport rates, indicating that small changes in placental glucose transporter content may have little or no effect on the rate of placental glucose transport (7,8). By late gestation, however, glucose transport capacity by the placenta has a dominant role in the regulation of glucose transport to the fetus (Figure 2, bottom panel) (10). On balance, however, by late gestation, the increasing gradient of glucose concentration across the placenta contributes only limited control over placental transport of glucose to the fetus (7).

B. Placental glucose transporters. Glucose uptake and transport by the endothelial, epithelial, and trophoblast cells of the placenta are mediated by facilitative transporter proteins, primarily Glut 1 (all species) and Glut 3 (rat, sheep, and variably reported to be present in human). Glut 8 also has been found in the sheep placenta, but has not been localized and its role in glucose transport is not known (13). Glut 1 appears to be the primary transporter and is found, accordingly, at both the maternal-facing microvillous trophoblast membrane and the fetal-facing basal trophoblast membrane (14). Glut 3 concentrations increase with gestation in sheep; Glut 3 is found primarily on the maternal-facing surface of the trophoblast where it might act specifically to take up glucose into the trophoblast cells themselves, thereby

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shown by the increasing slope of PGT as gestational age increases. Bottom panel: At mid gestation, most of PGT is due to the maternal-fetal glucose concentration gradient whereas by term, the placental transport capacity for glucose accounts for most of PGT. Reproduced with permission from Hay, W. W., Jr. Nutrition and development of the fetus: carbohydrate and lipid metabolism. In Walker, W. A., Watkins, J. B., Duggan C. P. (eds.), Nutrition in Pediatrics (Basic Science and Clinical Applications), 3rd Edition, BC Decker Inc Publisher, Hamilton, Ontario, Canada, pp 449–470, 2003 (10). Adapted from Molina RD, Meschia G, Battaglia FC, Hay WW Jr. Maturation of placental glucose transfer capacity in the ovine pregnancy. Am J Physiol 1991;261:R697–704 (11).

allowing bi-directional transport across the placenta to be regulated by Glut 1. Glut 4 also has been found in the human placenta, but not well localized and although recent studies have shown increased insulin-mediated glucose uptake in perfused human placentas from insulin-treated diabetic women and in first trimester chorionic villous explants (15), in mature placentas, there is no evidence that insulin regulates glucose uptake from either the maternal or the fetal circulation.

To date, there is no solid experimental evidence to quantify the relative role of each of these transporters in transplacental glucose transport, although changes in transporter abundance do vary directly with changes in transport capacity. For example, the differential localization of glucose transporter isoforms in the placenta might have some relation to directional glucose transport as well as its efficiency, as indicated by recent studies in the multilayered synepitheliochorial ruminant placenta in which Glut 1 is found at the uterine epithelium and on the fetal-facing basal membrane and Glut 3 on the apical surface microvilli (16,17). Thus, a glucose molecule must use both Glut 1 and Glut 3 in sequence to cross from maternal to fetal circulations (7). The distribution of Glut 3 has important implications for glucose flux across the placenta. Kinetically, the Glut 3 on the outer membrane facing the maternal blood is more efficient than the co-localized Glut 1 at maintaining glucose transport at low maternal blood concentrations (its K_m is two to five times lower). The very high affinity of the Glut 3 transporter also might serve to maintain glucose utilization by the trophoblast at the physiologically very low fetal glucose concentrations ($\sim$1.1 mmol/L in sheep vs. $\sim$3 mmol/L in humans). This might be a particularly important requirement given the high energy needs of the trophoblast to maintain its critical energy-dependent roles in active amino acid transport and ion (e.g., calcium) pumps. It also is worth noting that ruminants produce fructose from glucose via the aldose reductase pathway in the trophoblast cells of the placenta (18). Fructose enters the fetal circulation where it is used at modest rates via glycolysis for the same metabolic fates as glucose. Fructose is not transported by Glut 3. Thus, the localization of Glut 3 at the apical, microvillous surface of the trophoblast is consistent with previous studies that have shown little ($<$10%) transport of labeled fructose from the fetal to the maternal circulation (18).

C. Placental glucose transport in pregnancies with intrauterine growth restriction. A common characteristic of all pregnancies with intrauterine growth restriction (IUGR) is relative fetal hypoglycemia. This phenomenon produces a larger maternal-fetal glucose concentration gradient and thus helps to compensate for the reduction

in placental glucose transport (PGT) capacity and rate of glucose flux from the maternal to the fetal circulation that occurs because of the smaller IUGR placenta. There is considerable variability, however, in how the reduced glucose transport capacity comes about. In the model of placental insufficiency produced by maternal hyperthermia during pregnancy that we primarily use in our lab (19), reduced PGT capacity occurs by both a smaller placenta and a decreased number of glucose transporters. The latter probably occurs as a result of decreased placental membrane surface area, although a selective reduction of the concentration of transporters per surface area cannot yet be ruled in or out. Our studies in normally developing fetal sheep, for example, have shown late gestational development of reduced Glut 1 mRNA and protein, Glut 3 mRNA, and Glut 8 mRNA and protein levels (Regnault T, Limesand S, Anthony R, Hay WW Jr, unpublished results). In contrast, our studies with Jacqueline Wallace at the Rowett Research Institute in Aberdeen, Scotland, have shown that in her model of the adolescent pregnant ewe, in which pregnant sheep that are still early in their adolescent growth phase produce a smaller placenta and thus a smaller fetus when allowed to over eat and become obese, have shown normal weight-specific glucose transport capacity and no reduction in Glut 1 and 3 mRNA levels, indicating that there are likely no transporter deficiencies or selective reductions in placental trophoblast membrane surface area that are responsible for transport (20,21). In this model, therefore, the small size of the placenta *per se* is the major limitation to placental glucose transfer from mother to fetus, while the reduced transport capacity in the hyperthermic IUGR model also appears to include a component due to reduced glucose transporter levels independent of the surface area of the placental membranes.

III. Fetal glucose utilization

A. Effects of fetal plasma glucose and insulin on fetal glucose metabolism. The rate of fetal glucose metabolism (total utilization as well as the rate of fetal glucose oxidation) depends directly on the simultaneous interaction of fetal plasma glucose and insulin concentrations (22). In near-term fetal sheep plasma glucose and insulin concentrations act additively and simultaneously to enhance fetal glucose utilization and oxidation to CO_2 (22). Although both glucose and insulin act independently (i.e., additively) to increase glucose utilization and oxidation in the fetus according to saturation kinetics, the relative proportion of glucose oxidized during short-term 3- to 4-hour studies (about 55 per cent in fetal sheep) does not change significantly

over the entire range of glucose utilized. Thus, the principal metabolic effect of insulin in the fetus is to increase the permeability of insulin-sensitive cells to glucose, enhancing glucose uptake and utilization in general, and oxidation of glucose in proportion to uptake and utilization, rather than to differentially affect intracellular pathways of glucose metabolism. At least this is true on balance for the whole fetus; individual tissues and metabolic pathways may vary significantly in their responsiveness to insulin. Such a permissive role for insulin in fetal sheep is different from that in adult humans, in whom higher rates of glucose utilization are partitioned more into glucose storage (fat and glycogen) than into oxidation. The same may be true in the human fetus, which, like the human adult, has the capacity to synthesize fat to a greater extent than the leaner fetal sheep. In fetal sheep the disposition of non-oxidized glucose carbon is less certain.

B. Glucose transporters and glucose uptake and metabolism in the fetus. Both acute and chronic changes in fetal glucose and insulin concentrations have been studied in fetal sheep to address the interrelationships among absolute changes in glucose and insulin concentrations, duration of change in glucose and insulin concentrations, expression of glucose transporter concentrations, and glucose utilization rates in the fetus. In chronic studies lasting two to four weeks in late gestation, sustained hyperglycemia was associated with a progressive decrease to normal or subnormal insulin concentrations (23). Under these conditions there was a transient increase in brain Glut 1 but not Glut 3 concentrations, and a progressive decline in liver and adipose tissue Glut 1 and myocardial and skeletal muscle Glut 1 and Glut 4 (24). Further studies showed that the chronic hyperglycemia and reduction in Glut 4 correlated with the development of insulin resistance (25). In contrast, chronic hypoglycemia (produced by maternal insulin infusion) produced a decline in brain Glut-3, and increase in brain Glut-1, and a subsequent decline in liver Glut 1, but no significant change in insulin-sensitive myocardium, skeletal muscle, or adipose tissue Glut 1 or 4 concentrations (24). These time-dependent and tissue- and isoform-specific changes in response to altered circulating glucose and/or insulin concentrations showed that cellular adaptations in Glut 1 and Glut 3 are geared toward protecting the fetus from perturbations in substrate availability, and the adaptations in Glut 4 are geared toward development of insulin resistance.

IV. Fetal insulin secretion

The fetal pancreas develops in the late first to early second trimester, producing measurable insulin concentrations by mid-gestation.

There is a gradual increase in basal insulin concentration and glucose- and arginine-induced insulin secretion towards term (26).

A. Regulation by glucose concentrations. Fetal sheep develop down-regulation of glucose-induced and basal insulin secretion in the presence of chronic, sustained, marked hyperglycemia (23,25), but increased insulin secretion with pulsatile hyperglycemia as in humans (30). Hypoglycemia decreases basal and glucose-induced insulin secretion (27,28), perhaps a fundamental aspect of how intrauterine growth restriction with fetal hypoglycemia decreases fetal pancreatic development and insulin secretion capacity. Clearly fetal insulin secretion responds variably to changes in glucose concentration that are dependent on the absolute change in glucose concentration, its magnitude, and its pattern (Figure 3) (27).

B. Changes in fetal insulin secretion with intrauterine growth restriction. Human fetuses with severe intrauterine growth restriction (IUGR) have less pancreatic endocrine tissue and exhibit β-cell dysfunction, which may limit β-cell function in later life and contribute to their increased incidence of non-insulin dependent diabetes mellitus. In our maternal hyperthermia-induced ovine model of IUGR, plasma insulin concentrations in the IUGR fetuses were 64% lower at baseline and 77% lower after glucose stimulated insulin secretion (GSIS) (29). Modeling of changes in plasma insulin concentration over time during GSIS revealed deficits in the insulin secretion rate in the IUGR fetuses compared to controls, which also was shown for arginine-stimulated insulin secretion. Fetal islets, immunopositive for insulin and glucagon, secreted insulin in response to increasing glucose and potassium chloride (KCl) concentrations. Insulin release as a fraction of total insulin content was greater in glucose-stimulated IUGR islets; however, the mass of insulin released per IUGR islet was lower due to an 82% reduction in their insulin content. A deficiency in islet glucose metabolism was found in the rate of islet glucose oxidation at maximal stimulatory glucose concentrations (11 mmol/L). These data showed that pancreatic islets from IUGR fetuses have impaired β-cell stimulus secretion coupling, as a result of reduced glucose-stimulated glucose oxidation rates, insulin biosynthesis, and insulin content, despite normal to increased fractional rates of insulin release that results from a greater proportion of releasable insulin due to lower insulin stores (30).

We also determined in the maternal hyperthermia-induced ovine model of IUGR whether replication, apoptosis, and neoformation contribute to fetal pancreatic β-cell mass (29). At 90% of term gestation, IUGR fetal and pancreatic weights were 58% and 59%, respectively,

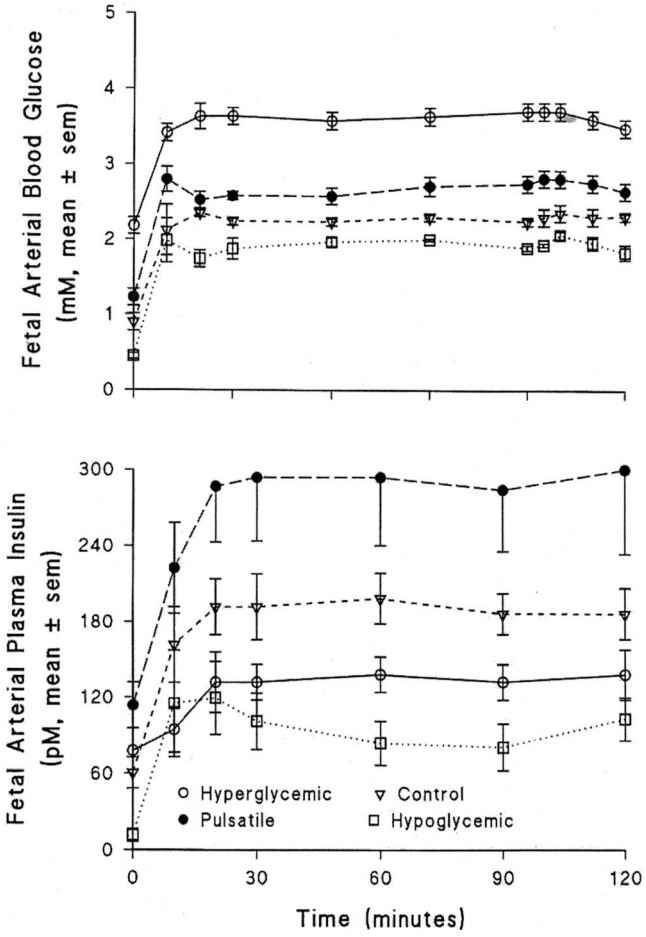

FIG. 3. Glucose stimulated insulin secretion as a function of sustained normal, increased, or decreased plasma glucose concentrations in fetal sheep (27). Four groups of fetal sheep were studied by hyperglycemic glucose clamp technique after each group had been maintained at a unique plasma glucose concentration for 12 days: ▽ = control fetuses; ○ = fetuses that were markedly and consistently hyperglycemic (about twice normal); • = fetuses that were mildly hyperglycemic but had 3 pulses of marked hyperglycemia lasting 60 minutes each during a 24 hour period; □ = Hypoglycemic fetuses that had a plasma glucose about 50% of normal. A. fetal arterial blood glucose concentrations in 4 groups of fetal sheep during a 120 minute hyperglycemic glucose clamp. Values from 30–120 min are significantly different ($P < 0.01$) among all groups. B: fetal arterial plasma insulin concentrations in the same 4 groups of animals during the 120 min hyperglycemic glucose clamps. Values are means ± SEM. Reproduced with permission from Carver et al. Am J Physiol 271 (Endocrinol Metab 34):E865–E871, 1996 (27).

less than pair fed controls. We identified a selective impairment of β-cell mass compared to other pancreatic cell types in IUGR fetuses. Insulin and insulin mRNA contents were less than other pancreatic endocrine hormones in the IUGR pancreases, as were pancreatic insulin positive area (42%) and β-cell mass (76%). Pancreatic β-cell apoptosis was not different between treatments. β-cell capacity for cell cycling, determined by proliferating cell nuclear antigen (PCNA) immunostaining, was not different between treatment groups. However, the percentage of β-cells actually undergoing mitosis was 72% lower in IUGR fetuses. These results indicate that in utero nutrient deficits decrease the population of pancreatic β-cells by lengthening G1, S, and G2 stages of interphase and decreasing mitosis near term. Based on these results, we are now setting out to test whether diminished β-cell mass in IUGR infants at birth, if not adequately compensated for after birth, might contribute to insufficient insulin production in later life and, thus, a predisposition to non-insulin dependent diabetes.

C. Changes in fetal insulin secretion with hypoglycemia. Because one common characteristic of fetuses with IUGR is relative hypoglycemia, we next considered whether fetal pancreatic adaptations to hypoglycemia alone, without other general aspects of IUGR, would, by itself, limit the capacity of fetal pancreatic β-cells to produce and/or secrete insulin. We measured glucose stimulated insulin secretion (GSIS) in normal control fetal sheep, fetal sheep made hypoglycemic by maternal insulin infusion for two weeks in late gestation and also determined whether a 5-day euglycemic recovery period (recovery fetuses) would restore insulin secretion capacity (28). Hypoglycemia significantly decreased plasma insulin concentrations in the hypoglycemic (0.13 ± 0.01 ng/ml) and recovery fetuses (0.11 ± 0.01 ng/ml); insulin concentrations returned to euglycemic control values (0.30 ± 0.01 ng/ml) in recovery fetuses (0.29 ± 0.04 ng/ml) during their euglycemic recovery period. Mean steady state plasma insulin concentration during GSIS study was reduced in hypoglycemic fetuses (0.40 ± 0.07 vs. 0.92 ± 0.10 ng/ml in controls); there was some recovery in the recovery group of fetuses (0.73 ± 0.10 ng/ml), but their mean insulin concentration during the GSIS period was still less than in the control group. Nonlinear modeling of GSIS showed that the time from onset of hyperglycemia until insulin concentration reached 50% of its final GSIS value (insulin secretion response time) was greater ($P < 0.01$) in both hypoglycemic (15.6 ± 2.8 min) and recovery fetuses (15.4 ± 1.5 min) than in control fetuses (6.3 ± 1.1 min). Insulin secretion responsiveness to arginine also was reduced by hypoglycemia (0.98 ± 0.11 ng/ml hypoglycemic vs. 1.82 ± 0.17 ng/ml controls, $P < 0.05$) and did

not recover with normalization of glucose concentration (1.21 ± 0.15 ng/ml recovery, $P < 0.05$ vs. control). In a further study of islets from hypoglycemic fetuses, we also observed increased islet insulin concentration, without an increase in the number of islets per pancreatic wet weight or the number of β-cells per islet (Limesand S, Hay WW Jr, unpublished data). In contrast to the IUGR fetuses from generalized placental insufficiency, therefore, late gestation chronic hypoglycemia did not develop a failure of β-cell growth or replication, but instead developed a defect in insulin secretion. This was independent of the capacity of these islets to take up and oxidize glucose, indicating that the defect in insulin secretion was likely downstream of early phases of glucose-insulin secretion coupling. These studies showed, therefore, that hypoglycemia itself could reduce fetal pancreatic insulin secretion responsiveness and that this inhibition, while not proven to be permanent, still showed delayed recovery, indicating a possible mechanism to account for a predisposition to Type 2 diabetes that could begin in fetal life.

V. Other aspects of glucose and amino acid metabolism in intrauterine growth restriction

Upregulation of mechanisms regulating glucose utilization. IUGR is a unique example of how fetal glucose metabolism and its regulatory mechanisms adapt to glucose deficiency and hypoglycemia to maintain normal fetal glucose metabolism. Regardless of the model (31), when the fetus is deprived of glucose by placental insufficiency or maternal hypoglycemia, fetal weight-specific glucose utilization rate is not very much different from normal rates (21). This indicates that part of the mechanisms to enhance glucose uptake by the fetus from the placenta is the production of further hypoglycemia, thereby increasing the maternal-fetal glucose concentration gradient. To do this, there must be an increase in the fetal tissue capacity for glucose uptake and/or utilization. This could come about by increased concentrations and/or activity and/or plasma membrane localization of glucose transporters, increased insulin signal transduction and thereby effectiveness to promote Glut 4 (and perhaps Glut 1) translocation to the cell membrane, or mechanisms of insulin metabolism into oxidative and/or non-oxidative pathways. In both fetal sheep and fetal rats with IUGR, Glut 1 and Glut 4 concentrations in myocardium, adipose tissue, and skeletal muscle do not decrease with sustained hypoglycemia (24,32,33), perhaps a positive adaptation to maintain glucose utilization despite hypoglycemia. It has not been determined if this

maintenance of glucose transporter concentration is sufficient by itself to maintain the glucose utilization at normal rates. We have, however, tested how well fetal sheep with placental insufficiency and IUGR could metabolize glucose at low plasma insulin concentrations (34). Fetal glucose metabolism was determined in IUGR fetuses, generated by exposing pregnant ewes to elevated ambient temperatures during the middle third of gestation, and pair-fed control fetuses near term at basal and hyperglycemic states. The Fick principle (measure of net flux of a substrate into or out of an organ, calculated for the whole fetus as umbilical blood flow rate times the umbilical venous-arterial blood glucose concentration difference) was used to calculate umbilical glucose uptake and, in conjunction with a ^{14}C-radiolabeled tracer of glucose, rates of fetal glucose utilization and oxidation. Glut 1 levels were determined by immunoblot in liver and brain tissues; Glut 1 and 4 were analyzed in skeletal muscle. Fetal results are presented in Table 1 with asterisks distinguishing significant differences at $P < 0.05$ between treatment groups at steady states (34). Relative amounts of Glut 1 per total protein were not different between control and IUGR fetuses for liver or skeletal muscle, but Glut 1 was greater in the brain of the IUGR fetus. Glut 4 levels also were not different between

TABLE 1

Glucose uptake, utilization, production, and oxidation rates in normal (control) and growth restricted (IUGR) fetal sheep under basal and hyperglycemic conditions. These data show that glucose utilization rate is maintained at control values in the IUGR fetuses under both basal and hyperglycemic conditions despite lower plasma glucose and/or insulin concentrations, thereby defining an adaptive increase in insulin action and glucose uptake capacity in the IUGR fetuses (= P < 0.05, IUGR values vs. control values) (34)*

Fetal values	Basal Study Period		Hyperglycemic Study Period	
	Control	IUGR	Control	IUGR
Plasma Glucose (mmol/L)	1.05 ± 0.06	0.63 ± 0.09*	2.31 ± 0.09	2.37 ± 0.09
Plasma Insulin (ng/mL)	0.26 ± 0.04	0.09 ± 0.01*	0.60 ± 0.10	0.17 ± 0.04*
Umbilical Blood Flow (ml/min/kg)	164.0 ± 7.3	126.0 ± 22.6*	152.3 ± 8.3	119.8 ± 5.7*
Umbilical Glucose Uptake Rate (μmol/min/kg)	25.0 ± 1.4	16.9 ± 4.1*	53.0 ± 2.6	57.9 ± 6.9
Glucose Utilization Rate (μmol/min/kg)	26.5 ± 2.4	28.1 ± 6.0	53.9 ± 2.8	53.3 ± 13.8
Glucose Production Rate (μmol/min/kg)	1.5 ± 1.6	11.2 ± 1.8*	0.9 ± 1.9	−4.6 ± 6.7
Glucose Oxidation Rate (μmol/min/kg)	16.7 ± 1.0	10.8 ± 1.5*	26.4 ± 1.6	23.7 ± 1.0

treatments in fetal skeletal muscle. Plasma glucose clearance rate, calculated as glucose utilization rate (IUGR) divided by the arterial plasma glucose concentration ([G]) for the IUGR fetuses (GUR/[G]) was not different from the control fetuses in the basal period, even though plasma glucose and insulin concentrations were significantly lower. Under hyperglycemic conditions, the predicted glucose utilization rates at the studied glucose and insulin concentrations indicated that fetal glucose utilization rate in the IUGR fetuses was 54% of control at basal and 81% at hyperglycemia. Other preliminary data (Anderson MA, Friedman J, Hay WW Jr, unpublished results) have shown increased levels of insulin receptor and reductions of the insulin signal transduction inhibitors, P85 protein subunit of phosphatidylinositol 3 kinase (PI3K, which negatively regulates the effect of insulin to promote Glut 4 translocation from inactive intracellular storage pools to active sites in the cell membrane where it enhances glucose uptake across the cell membrane) and glycogen synthase kinase (GSK, which negatively regulates glycogen synthase and the synthesis of glucose into glycogen). Therefore, fetal tissues had adapted to their hypoglycemic environment by developing mechanisms to promote glucose uptake and utilization, possibly via enhanced insulin action.

Downregulation of mechanisms regulating growth. In contrast to the upregulation of glucose utilization capacity, IUGR fetuses have as their principal manifestation a slower rate of growth. Perhaps the natural substitution of such a slower rate of growth allows normal cellular metabolism to continue when placental insufficiency deprives the fetus of normal rates of nutrient substrates for both metabolism and growth. Regardless of this teleological hypothesis, our data in fetal sheep regarding mechanisms that regulate the synthesis of amino acids into protein show the opposite of up-regulated glucose utilization capacity. Instead, for amino acid incorporation into protein, we have found decreased insulin signal transduction proteins, including mammalian target of rapamycin (mTOR), p70S6K, and eukaryotic initiation factor 4E (eIF4E) that regulate the the synthesis of amino acids into protein, plus an increase in the binding protein 4E-BP1 that would inhibit the function of eIF4E. Furthermore, after first showing that insulin selectively activates the mitogen activated protein kinase (MAPK) pathway in skeletal muscle and liver (35), more recently we also have found reduced skeletal muscle cell ERK-2 levels in IUGR fetuses (Friedman J, Regnault TRH, Hay WW Jr, unpublished data). These proteins regulate cell turnover and thus hyperplasia; thus, a decrease in their levels would indicate inhibition of cell growth. These

recent observations provide support for our hypothesis that the effect of insulin to promote cell turnover and hyperplasia is reduced at several regulatory steps.

Conclusions, future plans, and clinical relevance

The fetus has considerable capacity to adapt metabolically to acute and chronic changes in glucose supply by relatively common and understandable mechanisms. Fetal glucose metabolism, both oxidative and non-oxidative in relatively equal proportions, is dependent on additive effects of fetal plasma glucose and insulin concentrations, although there is little effect of basal insulin concentration on this metabolism. Glucose-stimulated insulin secretion increases over gestation, can be down-regulated by constant, high concentrations of glucose, but enhanced by pulsatile hyperglycemia. Insulin production is diminished in cases of intrauterine growth restriction (IUGR) by inhibition of pancreatic β-cell replication, but not by mechanisms that regulate insulin production or secretion, while the opposite occurs with just persistent hypoglycemia, despite its common occurrence in IUGR. More recent cell and molecular studies have shown that chronic hyperglycemia down-regulates both glucose tolerance and insulin sensitivity coincident with decreased expression of skeletal muscle and hepatic Glut 1 and 4 glucose transporters, while up-regulation of these transporters and selected insulin signal transduction proteins that regulate glucose uptake and utilization occurs with chronic hypoglycemia. The opposite occurs for insulin signal transduction proteins that regulate amino acid synthesis into protein. These studies demonstrate the mixed phenotype of the IUGR fetus that includes enhanced glucose utilization capacity, but diminished protein synthesis and growth.

Thus, the fetus has considerable capacity to adapt metabolically to acute and chronic changes in glucose supply by relatively common and understandable mechanisms. Current research is designed to test whether we can reverse these molecular and physiological changes in glucose utilization capacity by selective re-introduction of nutrient (glucose or amino acids, or both) and hormonal (insulin or IGF-1 or both) supplies, directly by infusion into the fetus or indirectly by infusion into the mother. Future studies are aimed at finding out whether such adaptations might lead to long term, possibly permanent changes in metabolic capacity that could underlie certain childhood and adult metabolic disorders such as insulin resistance, obesity, and diabetes mellitus. An additional goal is to reexamine the mechanisms

by which protein supplements to pregnant women often leads, surprisingly, to increased fetal morbidity and even mortality.

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DISCUSSION

Mackowiak, Baltimore: That was terrific Bill. I was interested in the use of hypothermia to create low birth weight. How do you create hypothermia in your sheep, and what's your hypothesis as to why birth weight is reduced?

Hay, Aurora: I wish I could have spent the day talking about the various models of intrauterine growth restriction. Hyperthermia occurs naturally, and the placental and fetal growth restriction that go with it occur naturally. This reproductive problem was first discovered by shepherds in North Australia and occurs all over the world where sheep that normally get pregnant in the springtime, have gone out of normal estrus cycle and developed their pregnancies in the hot summer months. Under these relatively hyperthermic conditions, the growth of the placenta is restricted, or runted, which, in turn, runts the fetus by limiting its nutritional supply. The placenta in this model is not just smaller, however. It also has limited transport capacity related to reduced nutrient substrate transporters on the placental membranes. We produce our laboratory model of hyperthermia in pregnant sheep in large environmental chambers where the pregnant sheep are exposed from early gestation to variable ambient temperatures controlled by computer to produce ~40° C (~104° F) for 12 hours during the day and then ~30° C (~86° F) for 12 hours during the night. The sheep eat ad libitum and the amount of food eaten each day is then pair fed to normothermic, control pregnant sheep in a portion of the laboratory kept at ~18°–20° C (~64°–68° F). The mechanisms responsible for how hyperthermia early in pregnancy restricts the growth of the placenta are not well understood. The growth restriction appears to begin over the first few days of exposure to hyperthermia, because we can determine whether an animal is a responder (will become hyperthermic and runt the placenta and fetus) or a non-responder (stays normothermic and produces a normal sized placenta and fetus) within the first three or four days. Mechanisms probably involve increased apoptosis and possibly decreased placental cell hyperplasia, but how these come about is not known. Subsequent reduction in placental blood flow results from the smaller size of the placenta, but also from altered placental vascular architecture along with reduced growth of terminal villi. We have no evidence that the hyperthermia affects the fetus differently from any other model; for example, the other model I showed you that didn't have changes in glucose transport capacity is an entirely different model produced by over feeding adolescent pregnant sheep, and they have exactly the same growth restricted fetal phenotype as does the hyperthermia model. So this is not a hyperthermic effect on fetal metabolism, just on placental growth.

Halsted, Davis: Thank you very much for your stimulating talk. Back in the late 1960's I had the occasion to participate in a research study in Egypt and saw many cases of kwashiorkor, or infant protein-calorie malnutrition. I don't know how well it's recognized, but these children all had significant hepatomegaly and what looked like portal hypertension with ascites. I imagine their liver disease could be similar to non-alcoholic fatty liver disease, which would go along with your concept of visceral adiposity. I wonder if you have any further comments about this possibility.

Hay: The visceral adipocity that we see involves both white and brown adipose tissue.

The livers and also the heart are enriched more in glycogen than they are in lipid; so this abdominal adiposity is similar to the kind you see in Type II diabetics.

Halsted: Could you comment further on my previous question about the development of obesity in the offspring of pregnant women who survived the Dutch famine winter of 1944?

Hay: Yes, the obesity that developed in some of these offspring, recognized as an increase in body mass index (BMI) as well as total body fat mass, was relatively specific to females whose mothers were undernourished from the famine in the first portion of gestation. That outcome has been recapitulated now in small and large animal models, so it appears to be a fairly consistent observation and biological effect of early undernutrition in pregnancy.

Boxer, Ann Arbor: Bill, what is the impact of malnutrition on insulin secretion? Is it altered? Is there more insulin elaborated per islet cell?

Hay: The reduction in fetal insulin secretion that we have seen in our hyperthermic IUGR model also has been seen in a variety of small animal models and in human fetuses with IUGR. The mechanisms are different among the models, however, and have been difficult to sort out. The fetal pancreas in the hyperthermic model is smaller, with fewer islets in some cases, but in almost all cases, fewer beta cells. The primary cellular defect is a reduced mitotic index; so in these IUGR fetuses the reduction in the capacity to secrete insulin is based on the development of fewer beta cells. The insulin secretion capacity per beta cell actually is normal or even increased. This contrasts with the chronic hypoglycemia model where pure hypoglycemia for periods of two weeks or more reduces insulin secretion capacity, perhaps by a defect at the level of exocytosis of insulin from the beta cell or from some kind of insulin trafficking defect within the beta cell.